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Plant uptake and translocation of air-borne chlordane and comparison with the soil-to-plant route

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Abstract

In order to assess fully the impact of persistent organic pollutants (POPs) on human health, pollutant exchange at the interface between terrestrial plants, in particular food crops, and other environmental compartments must be thoroughly understood. In this regard, transfers of multicomponent and chiral pollutants are particularly informative. In the present study, zucchini (*Cucurbita pepo* L.) was planted in containerized, uncontaminated soil under both greenhouse and field conditions and exposed to air-borne chlordane contamination at 14.0 and 0.20 ng/m^3 (average, greenhouses), and 2.2 ng/m^3 (average, field). Chiral gas chromatography interfaced to an ion trap mass spectrometer was used to determine the chiral (*trans*-chlordane, TC, and *cis*-chlordane, CC) and achiral (*trans*-nonachlor, TN) chlordane components in vegetation, air, and soil compartments. The chlordane components of interest were detected in all vegetation tissues examined—root, stem, leaves, and fruits. When compared with the data from a soil-to-plant uptake study, the compositional profile of the chlordane components, i.e. the component fractions of TC, CC, and TN, in plant tissues, showed significantly different patterns between the air-to-plant and soil-to-plant pathways. Changes in the enantiomer fractions of TC and CC in plant tissues relative to the source, i.e. air or soil, although observed, were not markedly different between the two routes. This report provides the first comprehensive comparison between two distinct plant uptake routes for POPs and their subsequent translocation within plant tissues.

Keywords: Persistent organic pollutants (POPs); Chlordane; Chiral pollutants; Plant uptake of atmospheric contaminants

1. Introduction

Persistent organic pollutants (POPs) (Wania and Mackay, 1996), such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), heptachlor, and technical chlordane, are characterized by their long half-lives in the environment (Mackay et al., 1997; Mattina et al., 1999; CA EPA, 2000). The vast majority of POPs enter the biosphere as a consequence of human activities: the application of synthetic pesticides to soil and vegetation; the unintentional output of polychlorinated dibenzo-*p*-dioxins (PCDD) from incinerators (Öberg and Bergström, 1986); and the accidental release of PCBs from transformers (ATSDR, 2000). Once introduced, environmental cycling of POPs, as well as their accumulation and magnification in biotic systems, are determined, in part, by their physicochemical properties (Iwata et al., 1993; Wania and Mackay, 1996; Ridal et al., 1997; Bidleman, 1999; Bidleman and Falconer, 1999; Jones and de Voogt, 1999; Wiberg et al., 2000).

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With vegetation covering more than 80% of the terrestrial portion of Earth (Ockenden et al., 1998), the role of plants, especially food crops, in pollutant transfers at the plant/air interface and the plant/soil interface is critical to assessing human health risks (McLachlan, 1996). In addition to the physicochemical properties of the pollutants, plant uptake and transport of organic xenobiotics will be affected by environmental conditions (e.g. temperature, humidity, soil properties) and specific issues related to plant physiology (Paterson et al., 1994; Simonich and Hites, 1995). At the soil/plant interface, weathered organic pollutants must first be mobilized from contaminated soil, transported across the root boundary, and translocated through the xylem into aerial plant tissues. At the air/plant interface, organic pollutants may either partition into the waxy cuticle of the leaves (Riederer, 1990) or transfer from air through the stomata, followed by translocation through the phloem (Barber et al., 2002).

Reports in the literature maintain that for lipophilic POPs (e.g., PCBs, PCDDs and PCDFs) the air-to-plant accumulation route dominates (McLachlan et al., 1995; Thomas et al., 1998). Nevertheless, a soil-to-plant uptake route has been explicitly demonstrated for some members of the *Cucurbitaceae* family, as well as some other food crops (Beall and Nash, 1971; Mattina et al., 2000, 2002; White, 2001). In particular, zucchini, a member of the *Cucurbitaceae* family, is especially vulnerable to uptake of chlordane and DDE via the soil-to-plant route (Mattina et al., 2000, 2002; White, 2001). On the other hand, the contribution, if any, of an air-to-plant uptake pathway for zucchini is unknown. The present study was designed to examine the air-to-plant uptake route for zucchini, *Cucurbitae pepo* L.

The organochlorine insecticide, chlordane, was the POP selected for this study both for consistency of comparison to previous studies (Mattina et al., 2000, 2002) and also because of its multicomponent and chiral properties. Introduced in the 1940s, chlordane was used as an agricultural pesticide on corn and citrus; as an insecticide and herbicide for home lawns and gardens; and as a termiticide around building foundations (Jantunen et al., 2000). The use and sale of chlordane were banned in the United States by the Environmental Protection Agency (USEPA) in 1988 (Dearth and Hites, 1991). Due to its persistence and toxicity, technical chlordane has also been listed as one of the 12 POPs subject to global treaty restrictions (Hogue, 2001). Technical chlordane is known to consist of 147 distinct components (Dearth and Hites, 1991), with *trans*-chlordane (TC), *cis*-chlordane (CC), and *trans*-nonachlor (TN) present in the highest amounts in the technical mixture (Sovocool et al., 1977). Furthermore, these three components persist longer in the environment than many of the minor components (Mattina et al., 1999). CC and TC are chiral molecules, present as racemates in the technical formulation. If their alteration in an environmental compartment is attributed to non-enantioselective processes (e.g., volatilization and solubilization), the enantiomer fraction (EF), defined in equation (1), where $C(+)$ and $C(-)$ are the concentrations of the (+)- and (−)-enantiomers respectively, should remain unchanged throughout the process.

$$\mathrm{EF}+ = \frac{C(+)}{C(+) + C(-)} \tag{1}$$

If the chiral pesticide interacts with a chiral substrate, as is often the case in biotic compartments, the process is enantioselective and a change in EF values will occur. Therefore, chiral pollutants can be tracked through both enantioselective and non-enantioselective processes from the source compartment into contiguous compartments (Bidleman and Falconer, 1999). In the studies reported herein, the absolute and relative concentrations of CC, TC and TN, and the enantiomeric profiles of CC and TC were determined so as to track the pollutants from their source compartment through plant tissues. This report is the first comprehensive study of the air-to-plant uptake of a POP by zucchini and the comparative examination of soil/plant/air uptake and transport phenomena.

2. Materials and methods

2.1. Greenhouse trials

Two greenhouses on the New Haven campus of the Connecticut Agricultural Experiment Station, each of which enclosed a different air-borne chlordane concentration, were utilized. In each greenhouse, two 22-gallon (61 cm × 47 cm × 51 cm) plastic containers were filled with a layer (3 cm) of marble chips, on top of which was placed fiberglass screening, and then approximately 62 kg of uncontaminated soil. The lid was secured on each container using duct tape and five 2.5 cm diameter holes were drilled in the lid top. Into four of the holes were inserted 2.5 cm diameter by 23 cm long PVC tubes, such that 5 cm extended above the top of the lid and the bottom of each tube penetrated 2.5–5 cm below the soil surface, and secured with duct tape. The fifth hole was used for irrigation and was plugged with a rubber stopper while not in use. Two drainage holes were drilled on the side of the bins at the level of the marble chips.

Zucchini seeds *Cucurbita pepo* cv "Black Beauty" (Seedway, Hall, NY, USA) were germinated on moist germination paper. A small amount of planting soil was

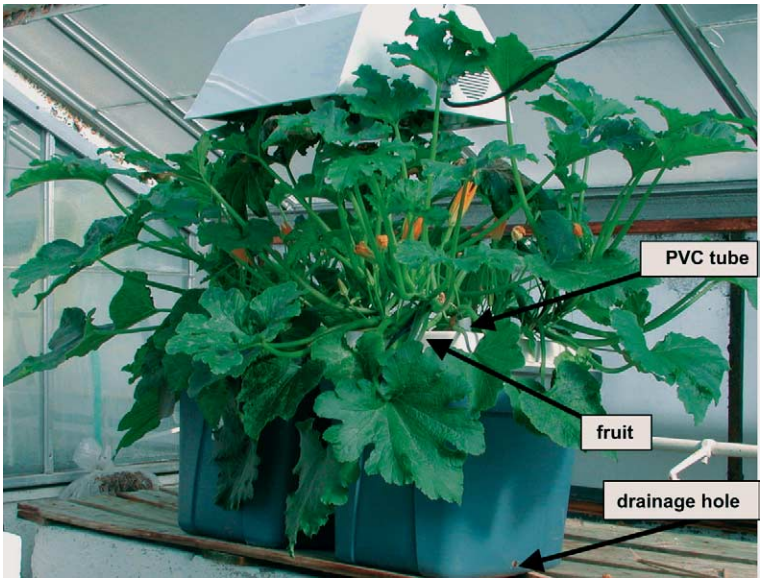

Fig. 1. Greenhouse setup for air-to-plant experiment.

tapped into each tube (to an approximate depth of 5 cm) secured to the bin lid and two germinated seeds were planted into this soil. Supplemental light from growth lamps (P.L. Light Systems, Canada) were used to achieve a 12 h day/night cycle. The experimental set up is shown in Fig. 1. Temperatures could not be coordinated and tightly controlled in the two greenhouses used in this study. However, continual temperature monitoring recorded in an adjacent greenhouse provided a temperature range assumed to be representative for both greenhouses used in this study.

Twenty-four hour air samples in the vicinity of the plants were collected on a weekly basis throughout the growing period with a high volume air sampler (HI-Q Environmental Products, San Diego, CA, USA) equipped with a glass-fiber filter preceding a polyurethane foam (PUF) plug. Rate of air flow through the plug was measured at the start and the end of the sampling period and averaged. PUFs were stored in clean glass jars at −20 °C until extraction.

2.2. Sample preparation and extraction

Duplicate extractions for each vegetation sample and each soil sample were performed using the procedures described below. Single extraction of each air sample was performed.

Soil. Four operationally defined soil fractions (White et al., 2002) were collected: pre- and post-growth *bulk soil* samples (top 30 cm); *near root* and *rhizosphere soil* samples were collected at the time of destructive harvest. All soil samples were air-dried overnight and sieved through a 2.5 mm mesh sieve to remove pebbles and to provide homogeneity. A previously described (Mattina et al., 1999) microwave-assisted extraction (MAE) method for soils was used. Briefly, 3 g of soil were mixed with 50 ml of 3:2 acetone:hexane, and spiked with 50 μl of internal standard solution containing 2 ng/μl of $^{13}C_{10}$-TN and 4 ng/μl racemic $^{13}C_{10}$-TC (Cambridge Isotope Laboratories, Andover, MA, USA) in toluene and subjected to MAE (CEM Corporation, Mathews, NC). The extract was then filtered, concentrated, and solvent exchanged to iso-octane prior to analysis.

Vegetation. Four zucchini tissues—root, stem, leaf, and fruit—were collected. Each tissue type was composited for each container in each of the greenhouses. All vegetation was rinsed thoroughly and chopped. Extraction procedures have been previously published (Mattina et al., 2002). In brief, 10 g of vegetation in a blender jar were mixed with 25 ml of iso-propanol, 50 ml of petroleum ether, and spiked with 50 μl of internal standard solution as described above. After blending, the solution was filtered, washed with water and saturated sodium sulfate, and cleaned-up on Florisil (U.S. Silica, Berkeley Springs, WV, USA).

Air. PUF plugs from the collected air samples were spiked with 25 μl of internal standard used for soil and vegetation samples, extracted in 350 ml of petroleum ether for 16 h in a Soxhlet apparatus, concentrated to less than 10 ml, and subjected to a Florisil cleanup. Details of this procedure have been previously published (Mattina et al., 2002).

2.3. Analysis and quantitation

Extracts were analyzed on a Saturn 2000 Ion Trap GC/MS system (Varian, Sugar Land, TX, USA) equipped with a 30 m×0.25 mm i.d.×0.25 μm film thickness γ-DEX-120 column (Supelco, Bellefonte, PA, USA). A deactivated silica column (0.5 m×0.25 mm) was attached before and after the analytical column with press-tight connectors (Restek, Bellefonte, PA, USA). The chiral γ-DEX-120 is a non-bonded phase column. The second deactivated silica column positioned after the analytical column was used so that the GC/MS interface could be maintained at a temperature which might produce excessive column bleed from the γ-DEX-120 into the ion trap. The GC oven was programmed as follows: initial temperature 120 °C, hold 1 min; ramped at 20 °C/min to 155 °C; ramped at 0.5 °C/min to 195 °C; ramped at 20 °C/min to 230 °C, hold 21.6 min. The injection port was maintained at 230 °C, and a 3 μl splitless injection was used. The mass spectrometer conditions were as follows: a 38 min filament delay, emission current 60 μA, target total ion current 5000 counts, maximum ionization time 25 000 μs, multiplier offset +200 V, and scan range m/z 345–425.

A set of calibration standards containing TC, CC, TN and oxychlordane (OXY), a chlordane metabolite, (ChemService, West Chester, PA, USA) were prepared in iso-octane: 10, 25, 50, 100, 250, 500, 1000 μg/l. Each solution consisted of racemic TC and CC at the cited concentration, TN at 1/2 the cited amount, and OXY at twice the cited value. Every calibration solution also contained 50 μg/l of each labeled component: (+)-$^{13}C_{10}$ TC, (−)-$^{13}C_{10}$ TC, and $^{13}C_{10}$ TN for internal standard calibration. For each instrument run of 24 samples, a complete set of standards was injected twice, once before and once after the sample extract injections. Each soil and vegetation extract was injected once, while air samples were injected twice. Iso-octane was injected after every 5–6 sample injections in order to prolong column lifetimes. Total chlordane is expressed throughout this paper as the sum of the ±TC, ±CC, and TN concentrations; the rationale for using this five-component sum has been previously discussed (Mattina et al., 1999).

PeakFit (SPSS, Chicago, IL, USA) was used to integrate extracted ion chromatograms. Principal component analysis (PCA) was performed using SYSTAT® (SPSS).

2.4. Quality assurance

Method blanks and standard reference soil (catalogue #720, Environmental Research Associates, Arvada, CO, USA) were analyzed periodically throughout the analysis of samples. As mentioned above, composite soil samples from *each* bin were extracted in duplicate; similarly, composite tissue samples from each bin were also extracted in duplicate. Should the relative standard deviation for the two extracts from a given sample exceed 25%, a third subsample was extracted. Air samples could only be extracted once. The internal standard quantitation method accounts for analyte losses through the extraction and clean-up steps. All chlordane concentrations in soil and vegetation were reported on a dry weight basis. Data reduction and data criteria have been previously described in detail (Eitzer et al., 2001; Mattina et al., 2002).

3. Result and discussion

3.1. Chlordane concentration in air and vegetation

The weekly total chlordane air concentrations in the two greenhouses during the course of the experiments are shown in Fig. 2; greenhouses were designated H (high, average total chlordane concentration 14.0 ng/m^3) and L (low, average 0.20 ng/m^3). The air concentrations in the two greenhouses differed significantly ($P = <0.001$) in spite of considerable fluctuation throughout the growing periods. In addition, ambient TC and CC in both greenhouses were close to racemic (0.51 and 0.51, respectively, in greenhouse H, and 0.50 and 0.51, respectively, in greenhouse L). The growing period was 66 days for the plants in greenhouse H and 51 days for those in greenhouse L. Estimated temperatures during the growth period averaged 19.1 °C in greenhouse H and 22.9 °C in greenhouse L.

Pre-growth bulk soil, post-growth bulk soil, near root, and rhizosphere soil for each bin were analyzed and the chlordane concentration in all soil samples was below the limit of quantitation (LOQ = 10 μg/l calibration standard or 5 μg/l for each enantiomer), and an approximate limit of detection (LOD) of <2 ng/g chlordane in soil. In spite of the non-quantifiable amounts in the soil, chlordane was detected in *all* tissue

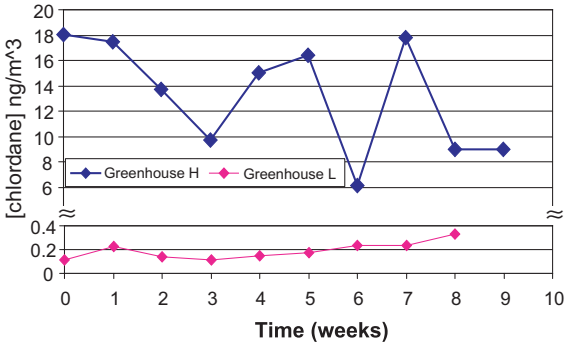

Fig. 2. Total chlordane concentration in greenhouse air at high (H) and low (L) levels throughout growing periods.

Table 1
Chlordane concentration in zucchini (ng/g) and in air (ng/m^3) in greenhouses H and L

	Biomass (g, dry weight)	Code[a]	(+)-TC	(−)-TC	(−)-CC	(+)-CC	TN	Conc. total[b]	Amount total[c]
Greenhouse H									
Air	–	**H**	3.4	3.3	1.9	1.9	3.4	14.0	
Leaf	29	H1	208.0	201.9	174.3	161.6	268.6	1014.3	29 415
	88	H2	38.5	36.4	31.1	28.3	49.4	183.6	16 157
Fruit	14	H1	93.6	98.0	78.6	91.5	130.3	492.0	6888
	28	H2	59.0	66.2	48.3	53.7	91.8	318.9	8929
Stem	6	H1	34.2	33.0	34.6	30.8	51.7	184.3	1106
	14	H2	51.4	48.2	46.3	41.8	62.4	250.1	3501
Root	2.5	H1	74.4	72.4	71.1	69.5	102.6	390.0	975
	3.6	H2	125.9	104.1	112.7	101.1	154.1	597.9	2152
Greenhouse L									
Air	–	**L**	0.04	0.04	0.03	0.03	0.06	0.20	
Leaf	157	L1	3.7	4.5	9.6	10.3	19.1	47.3	7426
	118	L2	5.2	6.5	11.0	13.0	22.7	58.4	6891
Fruit	88	L1	4.3	5.3	7.2	11.9	19.2	47.7	4198
	78	L2	2.9	4.4	7.4	10.6	12.2	37.5	2925
Stem	29	L1	18.7	21.2	50.1	42.3	112.2	244.5	7091
	22	L2	15.0	15.7	37.4	32.7	95.3	196.0	4312
Root	16	L1	20.6	20.4	50.3	46.4	136.5	274.2	4387
	15	L2	15.3	14.6	37.4	32.4	98.3	198.1	2972

[a] The designations "1" and "2" in the Code column represent the two bins in the H and L greenhouses.
[b] Sum = the sum of the concentrations of TC, CC, and TN.
[c] Total in ng, "Biomass" times "Conc. total".

samples, a result inconsistent with a soil-to-plant uptake route. The vegetation data from the duplicate bins in each greenhouse are summarized in Table 1. Although the concentrations (ng/g) and the biomass (ng) vary widely for the two replicates within each greenhouse, total chlordane (chlordane concentration × biomass = ng chlordane) in the tissue compartments from both replicate bins in each greenhouse was similar, as shown in the column "Amount Total". The chlordane concentration in the leaf tissue, the plant compartment assumed to be most impacted by an air-to-plant uptake route, is markedly higher in the H greenhouse versus the L greenhouse.

It is possible to determine the distribution pattern of the chlordane residues in the zucchini tissues from this greenhouse study using the following approach. We divide the ng chlordane for a particular tissue type, shown in the "Amount Total" column in Table 1, by the ng chlordane sum in all the vegetation from the given bin, e.g., LeafH1/(LeafH1 + FruitH1 + StemH1 + RootH1). In a similar fashion the pattern of chlordane distribution in the tissues from a field study, summarized in Table 2, may also be determined from the "Amount Total" column in Table 2. In the field investigation we studied in detail the uptake and translocation of soil-bound chlordane by zucchini. Briefly, in the field study, zucchini was grown in containerized soil, which was contaminated with weathered chlordane residues at four concentration levels ranging from below LOD up to 4633 ng/g. Samples in Table 2 are coded as HS, MS, and LS to represent samples from high, medium, and low levels of chlordane contaminated soil, and CS to represent uncontaminated soil. Because zucchini in the field bins grew largely within 60 cm of the ground surface, only data from air sampled in the field at the 60 cm height were included in the table. As may be seen in data from Table 2, chlordane was detected in all four tissue compartments, root, stem, leaf, and fruit, at all four levels of chlordane contamination. An increase in soil concentration resulted in higher chlordane concentration in all of the vegetation compartments examined, consistent with chlordane taken up by the root and translocated through stem to leaf and fruit. However, when we compare the distribution ratios, determined as described above, from the greenhouse versus the field studies, we note that greenhouse zucchini patterns are

Table 2
Chlordane concentration in zucchini (ng/g) and soil (ng/g) in the soil-to-plant field study

	Total mass (g, dry weight)	Code[a]	(+)-TC	(−)-TC	(−)-CC	(+)-CC	TN	Conc. total[b]	Amount total[c]
Field high soil									
Soil	112 580	HS1	803.9	883.2	926.0	1121.6	777.4	4512.0	507 960 960
	112 580	HS2	824.9	935.7	961.0	1121.4	789.8	4632.7	521 549 366
Leaf	350	HS1	545.8	581.3	704.1	776.3	231.2	2838.6	993 510
	339	HS2	315.5	368.5	441.8	491.6	142.0	1759.5	596 471
Fruit	338	HS1	212.2	230.0	223.1	336.1	85.3	1086.6	367 271
	357	HS2	184.1	189.5	168.6	271.7	82.8	896.7	320 122
Stem	79	HS1	1770.1	1622.4	2226.2	2266.1	863.3	8748.1	691 100
	79	HS2	1725.4	1644.1	2331.4	2201.3	843.9	8746.1	690 942
Root	14	HS1	6878.1	6172.2	8257.9	9129.7	5280.6	35 718.6	500 060
	25	HS2	4677.5	3953.3	5536.8	5785.8	3175.1	23 128.5	578 213
Field medium soil									
Soil	112 580	MS1	343.3	410.9	418.3	478.5	340.1	1991.1	224 158 038
	112 580	MS2	344.2	375.8	380.9	470.5	339.4	1910.8	215 117 864
Leaf	225	MS1	251.9	288.7	364.4	401.7	117.3	1424.0	320 400
	392	MS2	204.1	218.4	277.2	296.1	103.7	1099.4	430 965
Fruit	258	MS1	167.9	172.5	151.5	267.6	70.8	830.3	214 217
	416	MS2	138.5	154.2	151.1	230.3	65.2	739.3	307 549
Stem	69	MS1	1328.1	1273.3	1831.9	1750.0	674.8	6858.2	473 216
	88	MS2	868.0	775.7	1135.2	1052.8	491.7	4323.3	380 450
Root	17	MS1	4898.6	4738.6	6137.0	6490.4	4172.7	26 437.2	449 432
	16	MS2	3229.7	2978.1	4044.2	4219.8	2591.6	17 063.5	273 016
Field low soil									
Soil	112 580	LS1	53.9	64.8	65.8	76.2	62.5	323.1	36 374 598
	112 580	LS2	74.6	82.4	81.3	98.6	81.5	418.4	47 103 472
Leaf	163	LS1	45.1	62.3	64.1	68.6	34.6	274.6	44 760
	213	LS2	53.2	66.1	74.8	79.9	43.6	317.6	67 649
Fruit	107	LS1	111.5	127.9	133.5	183.5	78.0	634.4	67 881
	314	LS2	111.8	132.8	142.8	182.0	79.0	648.3	203 566
Stem	51	LS1	278.8	282.5	412.6	372.4	195.2	1541.5	78 617
	48	LS2	385.6	379.7	554.0	489.9	279.7	2088.8	100 262
Root	12	LS1	1754.4	1578.8	2247.1	2290.8	1675.1	9546.1	114 553
	13	LS2	1182.1	1140.8	1548.7	1654.2	1185.9	6711.5	87 250
Air (ng/m^3) 60 cm above ground			0.40	0.48	0.42	0.46	0.42	2.18	
Field clean soil									
Leaf	287	CS1	7.3	9.5	10.6	10.8	6.6	44.8	12 858
	254	CS2	6.0	6.7	5.8	7.7	9.6	35.7	9068
Fruit	238	CS1	26.0	30.5	31.5	36.1	30.2	154.4	36 747
	198	CS2	18.9	24.7	24.5	33.0	28.5	129.5	25 641
Stem	65	CS1	19.8	21.1	26.8	27.1	19.7	114.4	7436
	64	CS2	15.5	16.6	21.8	22.3	21.4	97.7	6253

Table 2 (*continued*)

	Total mass (g, dry weight)	Code[a]	(+)-TC	(−)-TC	(−)-CC	(+)-CC	TN	Conc. total[b]	Amount total[c]
Root	17	CS1	56.6	52.7	73.3	73.7	76.8	333.2	5664
	10	CS2	71.6	56.4	91.0	90.9	99.7	409.5	4095

[a] The designations "1" and "2" in the Code column represent the two bins in the field at the indicated level of soil contamination.
[b] Sum = the sum of the concentrations of TC, CC, and TN.
[c] Total in ng, "Biomass" times "Conc. total".

similar to those for zucchini grown in clean soil in the field, but *different from* those of zucchini grown in contaminated soil in the field. This comparison demonstrates in a qualitative fashion that zucchini uptakes, translocates, and accumulates at least one POP, chlordane, via an air-to-plant route, *in addition to* the remarkable bioavailability of weathered soil-bound chlordane previously noted for zucchini (Mattina et al., 2002).

The data from the present greenhouse study (Table 1) merit additional comments. First, chlordane is present at quantifiable amounts in *all* four plant tissue types examined. The apparent tendency of chlordane to accumulate in zucchini roots, regardless of the source compartment, awaits elucidation of the mechanism of transport of chlordane through the xylem and the phloem. Secondly, the chlordane metabolite, OXY, was among the compounds quantified. Contrary to the substantial concentrations of OXY found in fauna (Kucklick et al., 2002), in zucchini tissues OXY was either not detected or was present only occasionally at very low concentrations (ranging from LOQ to 46.4 ng/g). In the light of this observation it is unlikely that significant metabolism *in planta* occurs within the growing period for zucchini.

3.2. Comparison of soil-to-root versus air-to-leaf pathways

We have demonstrated previously (Mattina et al., 2000, 2002; White et al., 2002) that soil-bound chlordane enters plants via root uptake, followed by translocation to the shoot. Additional research from our laboratory has resulted in an empirical "dose in soil/uptake by zucchini" relationship (Lee et al., submitted for publication). In the discussion above we describe a qualitative comparison of the chlordane distribution patterns in zucchini tissues from the soil-to-plant versus air-to-plant regimes. Below we present several quantitative comparisons of the data from the two routes.

1. *Principal Component Analysis of CFs*

Component fractions (CFs), defined in Eq. (2), for TC, CC, and TN, were used for comparing the two uptake pathways.

$$\mathrm{CF} = \frac{\text{Concentration of a single component}}{[\mathrm{TC}] + [\mathrm{CC}] + [\mathrm{TN}]} \tag{2}$$

CFs (for each of the five components, +TC, −TC, −CC, +CC, TN) were determined for each of the plant tissue types. The CFs for root and leaf tissues from zucchini grown in the replicate bins in the H and L greenhouses (present study, Table 1) and in the field (Table 2) were subject to PCA. Factor (1) and Factor (2) plots are shown in Fig. 3A for roots and 3B for leaves and the component loadings and % total variance shown in Table 3. From Fig. 3A, it is apparent that roots from contaminated soil in the field experiment (HS, MS, and LS) group together and apart from the roots grown in clean soil in the field (CS) and in greenhouses, HA and LA. A similar but somewhat less compact grouping is noted in Fig. 3B for the leaf tissues. The conclusion is explicit that the CF patterns of the chlordane residues in zucchini root and leaf tissues differ for contamination via the two different source compartments, air and soil.

2. *Percent change in CFs in plant tissue versus chlordane source*

A second visualization of the difference between the two uptake routes is the CF patterns shown in the graphs of Fig. 4. In Fig. 4A the change in CF value in the tissue compartment versus that in the source compartment—air—was plotted for zucchini grown in each of the two replicate bins from the H and L greenhouses; and Fig. 4B shows the change in CF values in the zucchini tissues versus that in the source compartment—high, medium, and low chlordane contaminated soil—plotted for zucchini grown in contaminated soil in each of the two bins in the field. Several trends in the data merit discussion: (a) for each replicate in Fig. 4A, TN (orange bar) preferentially partitions into the leaf from the air. (b) For each replicate in Fig. 4B, TN preferentially attenuates in the root relative to the soil. (c) Both TC enantiomers (blue and yellow bars) attenuate in the leaf relative to the air as shown in Fig. 4A. While +TC (blue) tends to accumulate in the root relative to the soil, −TC (yellow) attenuates in the root compartment (Fig. 4B). (d) Both CC enantiomers (lilac and green bars) tend to accumulate in the leaf relative to the air, Fig. 4A, and in the root relative to the soil,

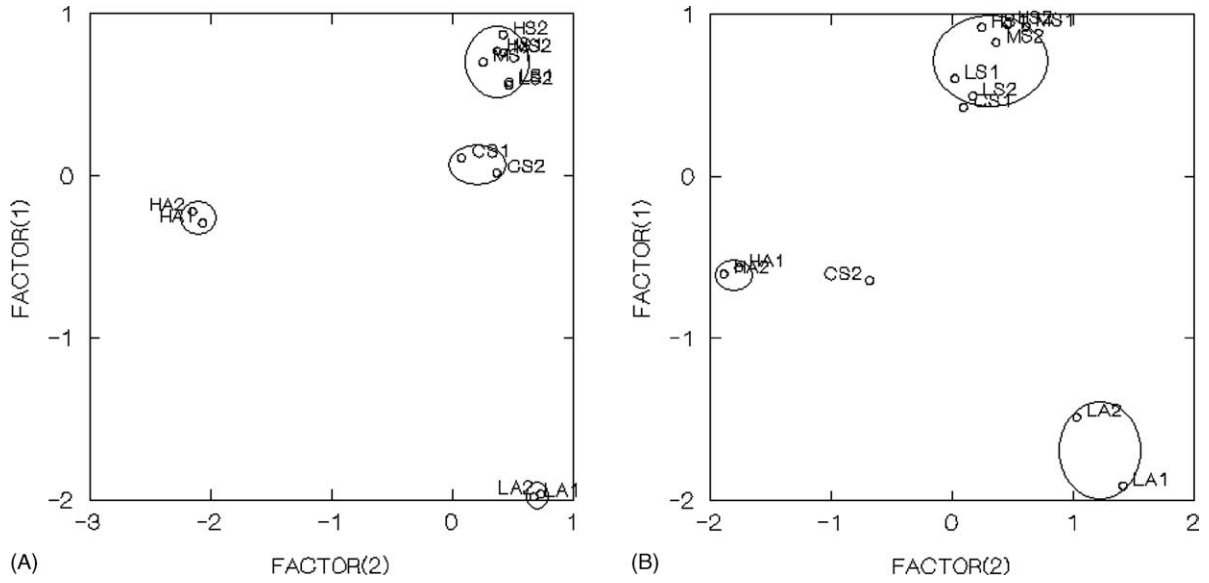

Fig. 3. (A) PCA plot for zucchini roots. Code description is as follows: HA, high chlordane contaminated air in greenhouse; LA, low chlordane contaminated air in greenhouse; CS, clean soil in bins in field; LS, low chlordane contaminated soil in bins in field; MS, medium chlordane contaminated soil in bins in field; HS, high chlordane contaminated soil in bins in field. (B) PCA plot for zucchini leaves. Code description is as in (A).

Table 3
Principal component analysis

	Roots		Leaf	
	Factor (1)	Factor (2)	Factor (1)	Factor (2)
+TC	0.884	−0.445	0.653	−0.732
−TC	0.888	−0.439	0.851	−0.476
−CC	0.848	0.519	0.860	0.466
+CC	0.879	0.465	0.675	0.717
TN	−0.998	0.065	−1.000	0.002
% Total variance	81.2	17.6	66.9	28.9

Fig. 4B. Furthermore, as is seen in Fig. 4B, accumulation of −CC (lilac) in the root is consistently larger than accumulation of +CC (green). The color coding of the graphs in Fig. 4 demonstrates once again that the two uptake routes, air-to-plant and soil-to-plant, produce consistently different CF patterns. Patterns for the zucchini grown in the clean soil in the two bins in the field versus the air at the 0.6 m height are similar to those shown in Fig. 4A.

3. *Enantiomer fractions*

Some of the patterns apparent in the graphs of Fig. 4 apply to the relative transport of enantiomer pairs. EFs, defined in Eq. (1), for CC versus TC were graphed for the source compartment, air, for the zucchini growing in the H and L greenhouses and in the clean soil in the field bin and shown in Fig. 5A. Although TC and CC in both H and L greenhouses was racemic, air at the 60 cm height in the field was not racemic. In Fig. 5B a similar graph is shown for the source compartment, soil, and the zucchini tissues grown in bins containing high, medium, and low chlordane-contaminated soil. Overlap of the circles enclosing leaf (yellow) and stem (red) tissues in Fig. 5A suggests that the enantioselectivity in moving from leaf to stem is relatively small. Similarly, in Fig. 5A the yellow circle also encloses the data for the source compartment, air, suggesting little enantioselectivity in moving from the air to the leaf. In Fig. 5B overlap is also noted in the circles enclosing root tissue (blue) and stem (red) tissue and the displacements along both ordinate and abscissa are small. What is obvious, however, is that

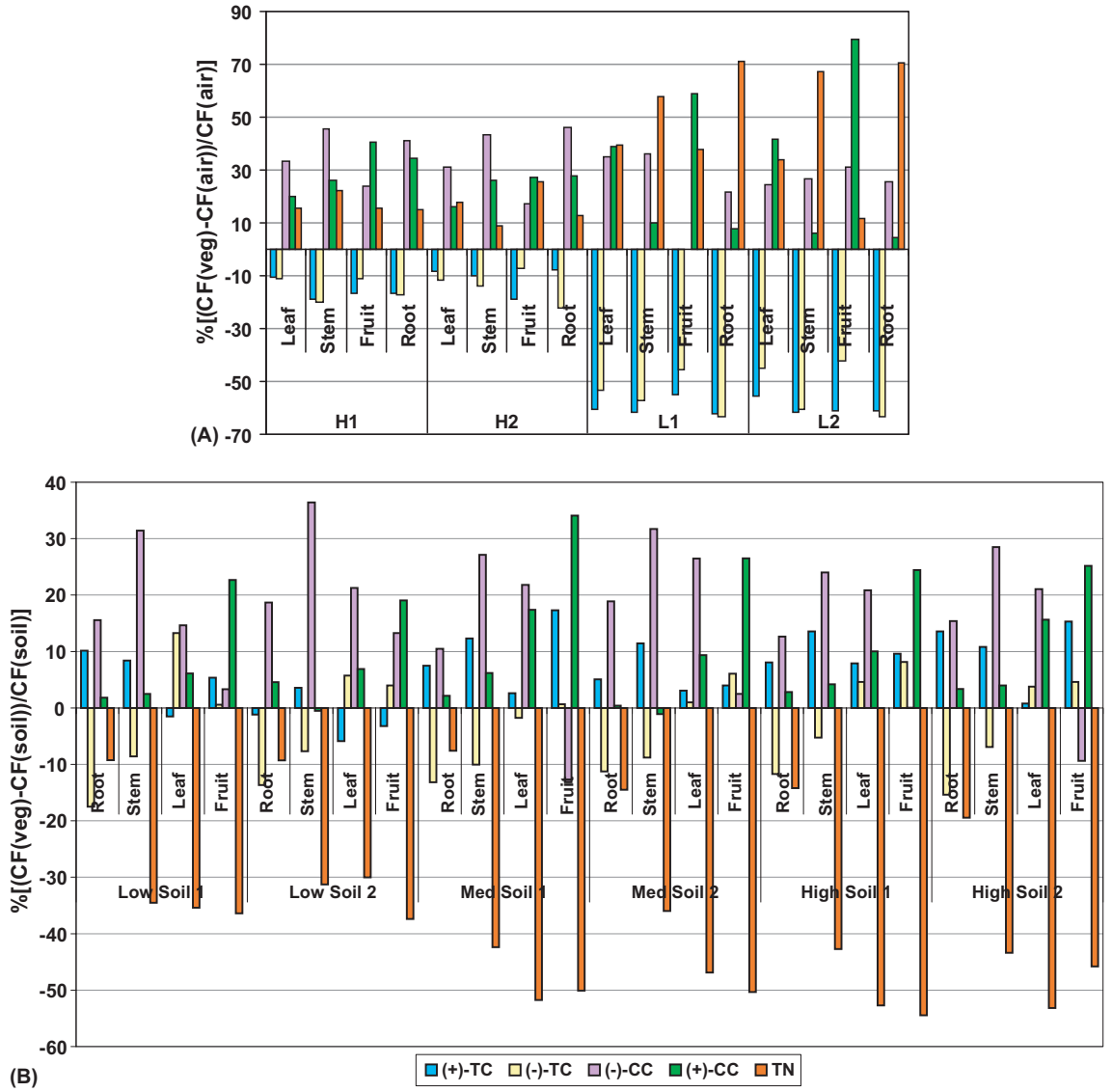

Fig. 4. (A) % Change in CF for zucchini vegetation relative to air-borne contamination in greenhouse. H1 and H2 data from vegetation in two bins exposed to high air-borne chlordane concentration; L1 and L2 data from vegetation in two bins exposed to low air-borne chlordane concentration. (B) % Change in CF for zucchini vegetation relative to soil-bound contamination; 1 and 2 represent two separate bins at low, medium, and high chlordane soil contamination level.

the EF patterns change substantially in moving from the source compartment, soil, into the root. The mechanisms responsible for transport of chlordane residues from the soil across the root boundary would appear to be enantioselective. The displacement of the yellow (leaf) and red (stem) circles in Fig. 5B also suggest an enantioselective transport process in translocating chlordane through the stem into the leaves. The enantioselective transport mechanisms implied by these data are under investigation.

The data from this study establish that all zucchini plant tissues are impacted by air-borne chlordane. This uptake route is in addition to that of the previously established uptake from weathered soil. Most significantly, it is clear from a qualitative argument and from three quantitative arguments that, for chlordane components, the patterns of uptake and transport for the two routes are readily distinguishable. The precise mechanisms and endogenous plant constituents responsible for the uptake and transport of these organic

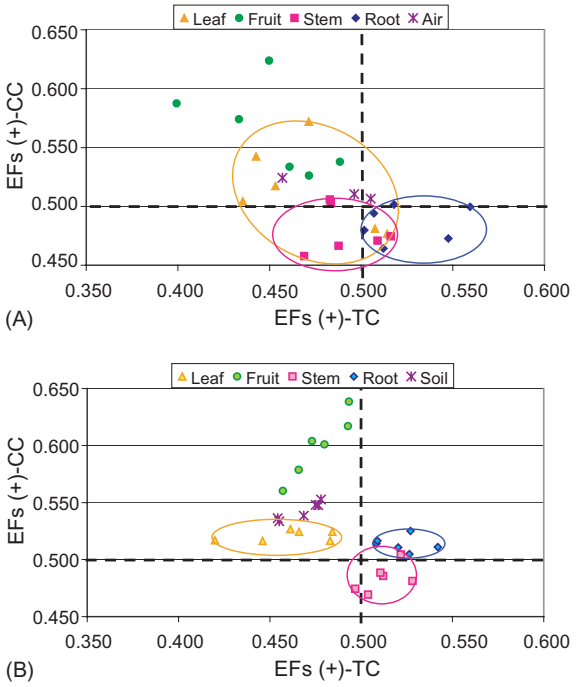

Fig. 5. (A) EFs of TC and CC for vegetation from greenhouse and field zucchini grown in clean soil with exposure to air-borne chlordane contamination. (B) EFs of TC and CC for vegetation from field zucchini grown in high, medium, and low chlordane contaminated soil.

pollutants through vegetative tissues are a topic of ongoing research.

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